

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

NAGASAWA, et al.

Appl. No. To be Assigned

Filed: Herewith

For: METHOD FOR DETECTING AND  
ISOLATING GENES

Art Unit: To be Assigned

Examiner: To be Assigned

Atty. Docket: 4001-0001C

**Preliminary Amendment**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination, please amend the application as follows:

**IN THE CLAIMS:**

Please replace claims 5, 6, 10, 11, 14, 15, 17, 21, 22, 23 and 29 with  
the following (respectively):

5. The vector of claim 1, wherein said gene inducing cell death under specific conditions is xanthine-guanine-phosphoribosyltransferase (gpt) gene.
6. A host cell transformed with the vector of claim 1.
10. The method of claim 8, wherein said test substance is a gene.
11. The method of claim 8, wherein said test substance is a low molecular weight compounds.

14. The method of claim 8, wherein said extracellular stimulation is stimulation by cytokine.
15. The method of claim 8, wherein said extracellular stimulation is a promoter region of interleukin 8 gene.
17. The method of claim 8, wherein said vector introduced into the cells of claim 6 is the vector of claim 4.
21. The method of claim 18, wherein said reporter gene is the luciferase gene.
22. The method of claim 18, wherein said extracellular stimulation is stimulation by cytokine.
23. The method of claim 18, wherein said extracellular stimulation is stimulation by tumor necrosis factor (TNF).
29. The host cells of claim 25, wherein said reporter gene is luciferase gene.

#### **REMARKS**

The above amendments are made to eliminate multiple claim dependency. A marked-up copy of these claim amendments is attached herewith.

Respectfully submitted,

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CLAIMS

1. A vector holding a gene capable of inducing cell death under specific conditions and linked downstream to a promoter region that functions in response to specific extracellular stimulation.

2. The vector of claim 1, wherein said extracellular stimulation is stimulation by cytokine.

3. The vector of claim 1, wherein said extracellular stimulation is stimulation by tumor necrosis factor (TNF).

4. The vector of claim 3, wherein said promoter region functioning in response to specific extracellular stimulation is a promoter region of interleukin 8 gene.

5. The vector of any one of claims 1 to 4, wherein said gene inducing cell death under specific conditions is xanthine-guanine-phosphoribosyltransferase (gpt) gene.

6. A host cell transformed with the vector of any one of claims 1 to 5.

7. The host cell of claim 6, wherein said cell cannot produce hypoxanthine-guanine-phosphoribosyltransferase (HGPRT), and said gene inducing cell death under specific conditions in the vector to be inserted is xanthine-guanine-phosphoribosyltransferase.

8. A method for detecting an inhibitory effect of a test substance on intracellular signal transduction, wherein the method comprises,

(a) introducing the test substance into or allowing the substance to act on the cells of claim 6,

(b) testing whether the cells obtained in (a) that the test substance

is introduced into or acts on are alive or not when the specific extracellular stimulation which causes said specific intracellular signal transduction under the specific conditions is added to the cells.

9. A method for detecting an inhibitory effect of a test substance on intracellular signal transduction, wherein the method comprises,

(a) introducing the test substance into or allowing the substance to act on the cells of claim 7,

(b) testing whether the cells that the test substance is introduced into or acts on obtained in (a) are alive or not when the specific extracellular stimulation which causes said specific intracellular signal transduction is added to the cells in the presence of 6-thioguanine.

10. The method of claim 8 or 9, wherein said test substance is a gene.

11. The method of claim 8 or 9, wherein said test substance is a low molecular weight compound.

12. A method for isolating a gene encoding a protein that inhibits specific intracellular signal transduction, wherein the method comprises,

(a) introducing a gene library into the cells of claim 6,

(b) screening living cells after the specific extracellular stimulation which causes said specific intracellular signal transduction is added under the specific conditions to the cells that the gene library is introduced into obtained in (a),

(c) isolating the gene introduced into said cells from the cells

screened in (b).

13. A method for isolating a gene encoding a protein that inhibits specific intracellular signal transduction, wherein the method comprises,

- (a) introducing a gene library into the cells of claim 7,
- (b) screening living cells after the specific extracellular stimulation which causes said specific intracellular signal transduction is added in the presence of 6-thioguanine to the cells that the gene library is introduced into obtained in (a),
- (c) isolating the gene in said cells from the cells screened in (b).

14. The method of [any one of] claims 8 [to 13], wherein said extracellular stimulation is stimulation by cytokine.

15. The method of [any one of] claims 8 [to 13], wherein said extracellular stimulation is stimulation by tumor necrosis factor (TNF).

16. The method of claim 15, wherein said promoter region functioning in response to specific extracellular stimulation is a promoter region of interleukin 8 gene.

17. The method of [any one of] claims 8 [to 13], wherein said vector introduced into the cells of claim 6 [or 7] is the vector of claim 4.

18. A method for detecting an inhibitory effect of a test gene on intracellular signal transduction, wherein the method comprises,

- (a) introducing into host cells a vector comprising a test gene that can be expressed in the host cells and a vector having a reporter gene downstream to a promoter region functioning in response to

specific extracellular stimulation,

(b) applying specific extracellular stimulation to the host cells obtained in (a) into which the vector is introduced and detecting the activity of a reporter gene product.

19. A method for detecting an inhibitory effect of a low molecular weight compound to be tested for intracellular signal transduction, wherein the method comprises,

(a) introducing into host cells a vector having a reporter gene downstream to a promoter region functioning in response to specific extracellular stimulation,

(b) allowing a low molecular weight compound to be tested to act on the host cells into which the vector obtained in (a) is introduced and detecting the activity of the reporter gene product.

20. A method for isolating a gene encoding a protein that inhibits specific intracellular signal transduction, wherein the method comprises,

(a) introducing into host cells a gene library that can be expressed in the host cells and a vector having a reporter gene downstream to a promoter region functioning in response to specific extracellular stimulation,

(b) applying specific extracellular stimulation to the host cells into which the vector in (a) is introduced, detecting the activity of the reporter gene product, and selecting cells in which said activity decreases,

(c) isolating a gene introduced into said cells from the cells screened in (b).

21. The method of [any one of] claims [18 to 20], wherein said

reporter gene is the luciferase gene.

22. The method of [any one of] claims 18 [to 21], wherein said extracellular stimulation is stimulation by cytokine.

23. The method of [any one of] claims 18 [to 21], wherein said extracellular stimulation is stimulation by tumor necrosis factor (TNF).

24. The method of claim 23, wherein said promoter region functioning in response to specific extracellular stimulation is a promoter region of the interleukin 8 gene.

25. A host cell transformed with a vector comprising a test gene that can be expressed in the host cells and a vector having a reporter gene downstream to a promoter region functioning in response to specific extracellular stimulation.

26. The host cells of claim 25, wherein said extracellular stimulation is stimulation by cytokine.

27. The host cells of claim 25, wherein said extracellular stimulation is stimulation by tumor necrosis factor (TNF).

28. The host cells of claim 27, wherein said promoter region functioning in response to specific extracellular stimulation is a promoter region of interleukin 8 gene.

29. The host cells of [any one of] claims 25 [to 28], wherein said reporter gene is luciferase gene.